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Neuropathology of Heavy Metal Intoxication

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The basis for utilizing altered morphology, i.e., pathology, in defining diseases of the nervous sytem is presented. The importance of recognizing the dynamics of pathologic processes is emphasized, particularly in understanding the pathogenesis of neural diseases. Demonstrative examples of the neuropathology of human heavy metal toxicity are presented. The limitations of descriptive pathology are considered, and the potentials of quantitative (morphometric) analysis for studying pathologic processes are introduced.

Introduction

Neurotoxicity may be an important feature of heavy metal intoxication. However, for us to recognize the neurotoxic effects of an agent, there must be a reproducible constellation of functional, biochemical, and structural alterations. Generally, we first recognize the dysfunction of the nervous system and then we search for the biochemical and structural correlates of the neural disorder. This report will be confined to a consideration of the morphologic changes, or so-called pathology of heavy metal neurotoxicity.

In defining a disease, we are looking for a reproducible pattern and evolution of the pathology. The analysis of the morphologic changes of a disease state is based upon identifying the elements affected, defining the changes the elements undergo, and determining the extent of these changes. Depending upon the structural level of analysis, the pathologic features may be identified at the level of the organelle, cell, and/or tissue. Finally, the sequence or pathogenesis of these alterations must be determined.

In terms of the nervous system, the number of cellular elements which must be identified and considered makes neuropathology appear unduely complex. However, at the level of the cell, neuropathology is not unique (*1*); neural elements undergo necrosis, injury, and repair comparable to cells in systemic tissues (*2*). Neuronal injury may be the result of a direct effect on the soma or secondary to axon injury. Neurons can also be affected by a reduced afferent input and undergo a form of secondary injury, so-called transsynaptic degeneration. Myelin degeneration may result from processes or agents directly affecting the myelin sheath or its cell of origin (the Schwann cell or oligodendroglia in the peripheral and central nervous systems, respectively). Demyelination also follows axon degeneration. The axon may be directly affected by an agent or process or indirectly affected by loss of its somal origin. The astrocyte is a highly sensitive cell which responds in a limited manner: astrocytes increase in numbers (astrocytosis) and/or also produce intracellular glial fibers (gliosis). The astrocyte generally responds by proliferation and formation of glial fibers except in cases of metabolic dysfunctions, when the cell responds by just nuclear enlargement and proliferation. Microglia are the central nervous system macrophages, and these elements respond by activation and phagocytosis. Blood vessels may be directly affected by agents or respond in a secondary manner to neural tissue injury. Irrespective of the genesis of the vascular changes, when capillaries or venules are affected, the blood-barrier systems are perturbed. Metabolic dysfunction and/or cerebral edema may result from the blood-brain barrier dysfunction. The neurocytopathologic consequences of cerebral edema include astrocytic and microglial reactions and in some instances demyelination. The topographic distribution of the lesions can be described in terms of central or peripheral nervous system,

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gray or white matter, regions of the brain, or specific nuclei. The analysis of the distribution of the lesions is important in comparing cases for determining the reproducible nature of the pathology.

In analyzing the observations, the dynamic nature of biologic reactions must be considered. The type of cellular reaction, i.e., primary or secondary, must be recognized. The dynamic nature of the changes must also be taken into account, insofar as the cell reactions may include a continuum of degeneration and repair. Moreover, other failing organs may produce secondary brain changes, i.e., metabolic encephalopathy. Age must also be considered; the developing nervous system responds differently than the mature system, and the lesions of aging must be distinguished from the effects of a toxin. The recognition of these complex interactions are important for understanding the pathogenesis of any neural toxin including heavy metals. Moreover, the nervous system is a multisystem organ, and this must be considered in understanding the pathogenesis of a disease. As we learn more about the nervous system, an analysis of the pathology in terms of functional units may provide a more rational basis for understanding the selective features of a given neurotoxin.

Neuropathology of Human Heavy Metal Toxicity

The cogent pathologic features of heavy metal neurotoxicity in man are presented in Table 1. These descriptions are brief notations of the general findings in man (*3*) and illustrate the diversity of neuropathologic findings caused by the different metals. Particular emphasis is also placed on the nature and distribution of the lesions.

Lead affects both the central and peripheral nervous systems of man (*3–5*). The peripheral neuropathy has long been recognized and characteristically effects the somatic motor nerves. The neuropathy in man is characterized by a loss of nerve fibers (axons and myelin sheaths), and to some degree segmental demyelination. Gray and white matter lesions are variable; proliferation of capillary endothelial cells and edema are the most consistent lesions reported. The vascular changes may be accentuated in the cerebellum. Neuronal necrosis is a variable finding and, when present, usually involves the neocortex and may be the result of the vasculopathy and anoxia. Astrocytes and microglia exhibit an activated state, and the most marked reactions are centered about small blood vessels, particularly in those cases with endothelial cell damage. Cases of encephalopathy with seizures and coma have been reported in which there are no discernible pathologic changes (*5*). Neurotoxicity is not a feature of cadmium toxicity (*6, 7*). Anosmia has been reported but these cases have been associated with very heavy dust environments (*7*). Arsenic compounds (*3, 8, 9*), irrespective of their molecular form, may produce in man an acute encephalopathy, and chronic exposure may lead to a neuropathy (*3–9*). The encephalopathy affects predominantly the white matter and is thought to be an allergic response (*8*). The neuropathy involves both sensory and motor fibers and is characterized by both axon and myelin degeneration (*9*). Concomitant with the motor neuron fiber degeneration is loss of anterior horn cells. Alkylmercury (*3, 10*) burdens produce in man a degeneration in both the central and peripheral nervous system. The neurotoxicity reflects a cumulative intoxication in which a minimal level is required prior to the onset of degeneration. The central nervous system changes involve the cerebellum, cerebral cortex, and basal ganglia. In the cerebellum there is a marked loss of internal granular layer neurons compared to the relative preservation of the Purkinje cells. The cerebral cortex exhibits not only a slight to moderate diffuse

Table 1. Neuropathologic features of human heavy metal toxicity.[a]

	Peripheral nervous system[b]		Central nervous system[b]			
	Sensory	Motor	Gray matter	White matter	Regions affected	Nuclei affected
Lead	0	+	+	+	Cerebellum	
Cadmium	0	0	0	0		
Arsenic	+	+	0	+		
Alkylmercury	+	0	+	0	Cerebellum	Granular layer
					Basal ganglia	Putamen
					frontal, parietal, and occipital lobes	Motor sensory visual
Manganese	0	0	0	0	Basal ganglia cerebellum	Globus pallidus

[a] The features are a compendium of multiple reports and constitute the most consistent findings observed.
[b] 0, abnormalities absent; +, abnormalities present.

loss of neurons but a selective marked neuron loss in the visual cortex, and a moderate neuronal loss in the precentral motor and postcentral sensory cortex. The basal ganglia exhibit a loss of neurons particularly in the putamen. The peripheral neuropathy is most prominent in the sensory system and is characterized by axon and myelin degeneration. The neurotoxicity of inorganic mercury is comparable to that of alkylmercury (*3*). Manganese toxicity (*3, 11, 12*) in humans affects the basal ganglia and to some degree the cerebellum. The basal ganglia change is characterized by an appreciable loss of neurons in the medial aspect of the globus pallidus. This lesion of the basal ganglia is a consistent observation. Less frequent is a reginal or lobularlike loss of both the Purkinje cells and internal granular layer neurons of the cerebellum. Peripheral neuropathy is not a feature of manganese intoxication.

Morphometric Approach to Neuropathology

The above neuropathologic descriptions are from overt cases of toxicity. The reports were based upon obvious changes, i.e., discernible loss of neurons, myelin, and/or axons and with an astrocytic reaction. However, throughout the literature there are cases of heavy metal burdens with clinical evidence of neurotoxicity but with no discernible pathologic changes. Such cases, of course, can be described in terms of biochemical lesions. The structural correlates and basis for the disease may not be apparent to a qualitative morphologic approach. A quantitative or morphometric analysis of these cases may be more revealing because the pathologic changes may not be in terms of a structural alteration but in a change in the numbers or proportions of critical elements. The morphometric studies are based upon applying stereologic procedures to tissue sections in a defined and coordinated manner (*13*). The methods may be applied to light and electron microscopic studies (*13*). Morphometric studies provide information which may then be subjected to statistical analysis. The morphometric studies can be applied to clinical material and have been widely used in analyzing nerve biopsies (*14*). Autopsy material can be studied at the cellular level; however, the artifacts intrinsic to such material limit quantitative studies at the ultrastructural level.

Morphometric analyses have been applied to a number of toxicologic problems. In our laboratory we utilized this approach to distinguish between the effects of lead intoxication and undernutrition on the developing brain (*15–18*). In brief, lead intoxication was produced in newborn rats by the Pentschew-Garro method (*19*). Suckling rats are initially exposed to lead via the milk and later exposure is to the feed shared with the mother. The lead in the diet affects the mother's nutrition, and this raises the question of which are effects of lead and which effects are related to nutrition? Suckling rats were undernourished by removing the nursing dam from her litter for increasing periods of time each day. The treated (lead and undernourished) pups and age-matched naive control pups were sacrificed at 30 days of age, and morphometric analyses were undertaken in a defined region of somatosensory cortex or pyramidal tract in the medulla. The morphometric analyses demonstrated that there are distinct and significant differences between the effects of lead toxicity and of undernutrition on the postnatal development of the rat nervous system. Astrocytic proliferation is retarded in the undernourished rat compared to the lead-treated rat

Table 2. Quantitative analysis of somatosensory cortex of 30-day rats.

	Control	Lead	Undernourished
Glia $\times 10^{-4}$ per mm^3	8.21	9.43	6.10
Neurons $\times 10^{-4}$ per mm^3	7.05	8.98	10.1
Neuropil, % of cortex	66	57	63
Synapses $\times 10^{-8}$ per mm^3	6.17	5.81	4.57
Synapses per neuron $\times 10^{-3}$	8.75	6.46	4.31

Table 3. Morphometric features of an average terminal bouton in molecular layer of 30-day rats.

	Control	Lead	Undernourished
Axial ratio	0.66	0.68	0.80
Volume, μm^3	0.17	0.18	0.29
Total surface, μm^2	4.9	5.0	7.6
Dense line surface, μm^2	0.93	1.0	1.0

Table 4. Morphometric analysis of myelin and axons of 30-day rats.[a]

	Control	Lead	Undernourished
Myelinated fibers, %	43	41	34
Promyelinated, % of myelinated axons	0.3	0.3	4.5
Mean number of myelin lamellae per myelinated axon	8.9	7.8	6.3
Width of myelin lamella, nm	10.7	11.7	10.7
Mean diameter of myelinated axons, μm	1.64	1.52	1.54
Diameter of axon when initial myelin lamella appears, μm	0.41	0.32	0.44

[a] Values based upon the analysis of the pyramidal tract in the medulla of three control, three lead, and three undernourished rats.

Table 5. Quantitative analysis of caudate nucleus of 60-day rats.[a]

	Dosage, mg/kg Pb-day		
	Control	25	200
Neuron $\times 10^{-5}$ per mm^3	1.38	1.27	1.18
Glia $\times 10^{-5}$ per mm^3	4.41	3.57	3.37
Boutons $\times 10^{-8}$ per mm^3	4.52	3.90	2.94
Synapses per neuron $\times 10^{-3}$	3.32	3.07	2.52

[a] Rats were dosed by gastric gavage with 0, 25, or 200 mg Pb/kg-day from day 2 through day 30 of life and were maintained on a "lead-free" diet for the next 30 days.

(Table 2). The form of the synaptic boutons is different in the undernourished pups (Table 3); while the boutons are larger in the undernourished rat, the surface area of contact points, i.e., the membrane densities, are the same in all three groups. Myelination is retarded in the undernourished rat; properly sequenced hypomyelination was observed in the lead-treated rat (Table 4).

The Pentschew-Garro lead model and the undernutrition model are extreme paradigms for lead toxicity and malnutrition; yet it required a quantitative study to elucidate the full range of differences. We have applied a morphometric analysis to a low level of lead toxicity in which the changes are more subtle (*20, 21*). Suckling rats were exposed to defined lead burdens for the first 30 days of life. The pups were kept on a normal, relatively lead-free diet for the next 30 days and were sacrificed at 60 days of age. A morphometric analysis of a predefined region of caudate nucleus was undertaken at the cellular and ultrastructural level. The results demonstrated an effect on postnatal neuron development and synaptogenesis (Table 5). The lead effects also appear to be dose-dependent.

Neuropathologic studies of toxicants such as heavy metals are dependent upon recognition of cellular changes and altered ultrastructure. Traditionally, these studies have been based upon recognizing qualitative differences; however, few studies have attempted to define in a quantitative manner these differences. Contemporary neuropathologic studies, particularly those related to neurotoxicology, will have to include objective quantitative structural analyses which can be subjected to statistical analysis. Such morphometric studies are necessary not only for demonstrating and defining the subtle undue burdens of toxicants but for demonstrating the full range of the effects. Quantitative base-line data are needed for determining the potential for regeneration and distinguishing between aging and toxin effects.

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